IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Carol W. Readhead and Robert Winston
Serial No. Unassigned
Filed: November 12, 2001
For: IN VITRO TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENE THERAPIES

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF EXPRESS MAILING

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November 12, 2001 — EL 900 688 451 US
DATE OF DEPOSIT — SIGNATURE: ANN WEISS — EXPRESS MAIL LABEL NO.

Dear Sir or Madam:

This Preliminary Amendment is filed with a divisional application of pending U.S. Serial No. 09/191,920, filed November 13, 1999. The divisional application filed herewith is directed to the subject matter of Claims 112, as originally filed in parent U.S. Serial No. 09/191,920, which claim was designated Group IV in a restriction requirement, mailed March 24, 2000. The Examiner is respectfully requested to consider the following amendments and remarks.

AMENDMENT

A Version With Markings To Show Changes Made is included beginning at page 5, after Applicant's Remarks.

IN THE SPECIFICATION:

In the Title, at page 1, lines 1-3, please delete the entire title, and insert therefor:

--IN VITRO TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES--.

At page 1, line 4, please delete the entire one-sentence paragraph, and insert the following:

--This application is a division of U.S. Non-provisional Application No. 09/191,920, filed on November 13, 1998, which claims the benefit of U.S. Provisional Application No.

60/065825, filed on November 14, 1997. This application is also related to U.S. Serial No._____, filed on November 12, 2001, U.S. Serial No._____, filed on November 12, 2001, and U.S. Serial No._____, filed on November 12, 2001, which are all divisions of U.S. Serial No. 09/191,920. This application is also related to U.S. Serial No. 09/272,443, filed March 19, 1999, which is a continuation of 09/191,920.--.

At page 4, line 14 through page 15, line 1, please delete the entire paragraph, and insert therefor the following:

--This invention also relates to a novel method for the isolation of spermatogonia, comprising obtaining spermatogonia from a mixed population of testicular cells by extruding the cells from the seminiferous tubules and gentle enzymatic disaggregation. The spermatogonia or stem cells which are to be genetically modified, may be isolated from a mixed cell population by a novel method including the utilization of a promoter sequence, which is only active in cycling spermatogonia stem cell populations, for example, b-Myb or a spermotogonia specific promoter, such as the c-kit promoter region, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, or FRMI (from fragile X site) promoter, optionally linked to a reporter construct, for example, the Green Fluorescent Protein Gene (EGFP). These unique promoter sequences drive the expression of the reporter construct only in the cycling spermatogonia. The spermatogonia, thus, are the only cells in the mixed population which will express the reporter construct and they, thus, may be isolated on this basis. In the case of the green fluorescent reporter construct, the cells may be sorted with the aid of, for example, a FACs scanner set at the appropriate wavelength or they may be selected by chemical methods.--.

At page 10, lines 11-17, please delete the entire paragraph and insert therefor the following:

--"Gene delivery (or transfection) mixture", in the context of this patent, means selected genetic material together with an appropriate vector mixed, for example, with an effective amount of lipid transfecting agent. The amount of each component of the mixture is chosen so that the transfection of a specific species of germ cell is optimized. Such optimization requires no more than routine experimentation. The ratio of DNA to lipid is

broad, preferably about 1: 1, although other proportions may also be utilized depending on the type of lipid agent and the DNA utilized. This proportion is not crucial.--.

At page 20, lines 15-22, please delete the entire paragraph and insert therefor the following:

--The GFP DNA-transferrin-polylysine viral complexes, prepared as described in Example 4 above, were delivered into the seminiferous tubules of three (3)-week-old B6D2F1 male mice. The DNA delivery by transferrin receptor-mediated endocytosis is described by Schmidt et al. and Wagner et al. (Schmidt et al., Cell 4: 41-51 (1986); Wagner, E., et al. PNAS (1990), (USA) 81: 3410-3414 (1990)). In addition, this delivery system relies on the capacity of adenoviruses to disrupt cell vesicles, such as endosomes and release the contents entrapped therein. The transfection efficiency of this system is almost 2,000 fold higher than lipofection.--.

IN THE CLAIMS:

Please cancel Claims 1-134, without prejudice, as originally filed with parent application 09/191,920, and add the following new Claim 135 as being directed to the subject matter of designated claim Group IV, which is herein elected.

--135.(New) A gene therapy method, comprising the steps of:

obtaining a male germ cell from a non-human vertebrate, said germ cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

transfecting the male germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker;

allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell; and

introducing said transfected male germ cell into the testis of a recipient vertebrate, wherein the polynucleotide encoding a gene product is derived from the same vertebrate species as the recipient vertebrate.--.

REMARKS

Applicant's Preliminary Amendment is submitted together with a divisional application directed to the subject matter of Claim 112, as originally filed in pending parent U.S. Serial No. 09/191,920, which claim was designated Group IV in a restriction requirement, mailed March 24, 2000.

The amendment of the title (at page 1, lines 1-3), is to bring these into conformity with the new Claim 135.

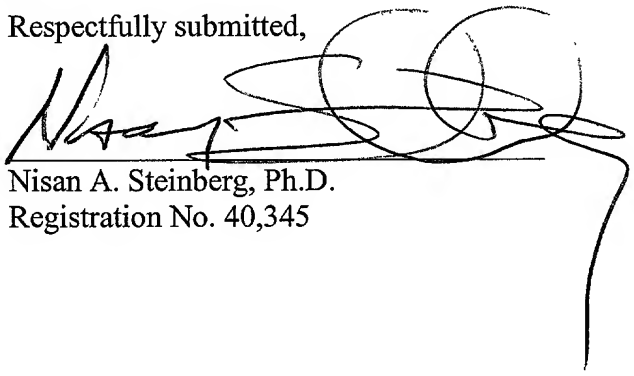
Applicant believes that no new matter is introduced by any amendments made herein.

At page 1, line 4, Applicant has added continuing data explaining the relationship to U.S. Serial No. 09/191,920 and other divisions and continuations thereof.

Applicant's cancellation of Claims 1-134 is made without prejudice. New Claim 135 is added. Support is found, e.g., in Claim 112, as originally filed.

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

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introducing said transfected male germ cell into the testis of a recipient vertebrate, wherein the polynucleotide encoding a gene product is derived from the same vertebrate species as the recipient vertebrate.--.

P07 41794

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Date: March 19, 1999

In re application of: Readhead et al.
Serial No. 09/191,920
Filed: November 13, 1998
For: TRANSFECTION STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES
Examiner: Unassigned
Unit: 1643

PRELIMINARY AMENDMENT

Assistant Commissioner for
Patents
Washington, D. C. 20231

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO THE ASSISTANT COMMISSIONER FOR PATENTS WASHINGTON, D C 20231, ON March 19, 1999
DATE
BY
ANN WEISS
March 19, 1999
(DATE OF SIGNATURE)

Dear Sir:

In connection with the above-captioned application, please enter the following amendments:

In the Specification:

Page 10, line 13, change "transfection agent" to --transfecting agent--.

Page 20, line 18, change "Schmit" to --Schmidt-- in the two places it appears.

In the claims:

Please cancel claims 126 through 130 without prejudice.

Please amend the following claim as follows:

114. (Amended) A kit for the transfection [and storage] of a male vertebrate's germ cells, [comprising] containing the component(s) of a transfection mixture, [said transfection mixture] comprising at least one transfecting agent, and optionally a genetic selection marker, whereby said kit may be used to transfect [and store] said germ cells [in a viable condition].

Request is made for correction of inventorship under 37 C.F.R. §1 48(b), deleting the name of OUTI HOVATTA.

Request has also been made to the Application Processing Division's Customer Correction Branch to place Carol W. Readhead, Pasadena, CA as the first inventor, followed by Robert Winston, London, United Kingdom, as they appeared on the cover page of the application filed November 13, 1998.

REMARKS

The amendment at page 10, line 13, is supported, for example, at page 10, lines 18 and 22.

The amendment to claim 114 is supported, for example, at page 5, lines 25-28

The amendment at page 20, line 18 is to correct a typographical error.

Deletion of **OUT HOVATTA** from this application is made necessary by the cancellation of claims 126 through 130 (and the amendment to claim 114). Dr. Hovatta is not a co-inventor of the remaining claims.

This is also stated in the accompanying petition filed in compliance with 37 C.F.R. § 1.48(b)(1). Payment of the requisite fee under 37 C.F.R. 1.17(i) is made herewith in compliance with 37 C.F.R. § 1.48(b)(2).

Therefore, a first office action not having been received, applicants respectfully request that these preliminary amendments be entered.

Respectfully submitted,

PRETTY, SCHROEDER & POPLAWSKI, P.C.

By: _____
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